

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/EP2004/008390

International filing date (day/month/year)
27.07.2004

Priority date (day/month/year)
01.08.2003

International Patent Classification (IPC) or both national classification and IPC
C12P13/08, C12R1/19

Applicant
DEGUSSA AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:



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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2004/008390

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
 - ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
 - ☒ a sequence listing
 - ☐ table(s) related to the sequence listing
 - b. format of material:
 - ☒ in written format
 - ☒ in computer readable form
 - c. time of filing/furnishing:
 - ☒ contained in the international application as filed.
 - ☒ filed together with the international application in computer readable form.
 - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2004/008390

Box No. II Priority

1. ☒ The following document has not been furnished:

☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☐ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-33,35-38
	No: Claims	34
Inventive step (IS)	Yes: Claims	
	No: Claims	1-38
Industrial applicability (IA)	Yes: Claims	1-38
	No: Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement (Continuation)

2.1 CITATIONS

Reference is made to the following documents:

- D1:** DEBABOV V G: "The threonine story", ADVANCES IN BIOCHEMICAL ENGINEERING, BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 79, 2003, pages 113-136
- D2:** DEBABOV V: "Construction of strains producing L-threonine", PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON THE GENETICS OF INDUSTRIAL MICROORGANISMS, TOKYO, JAPAN, 1982, pages 254-258
- D3:** US-A-6 025 169 (KOYAMA YOSUKE ET AL) 15 February 2000
- D4:** EP-A-0 796 916 (TRIPLE A B V) 24 September 1997
- D5:** SHIU-LAN L ET AL: "Study on the microfiltration of Escherichia coli-containing fermentation broth by a ceramic membrane filter", JOURNAL OF MEMBRANE SCIENCE, vol. 110, no. 2, 21 February 1996, pages 203-210
- D6:** DE 101 03 778 A (DEGUSSA) 14 March 2002
- D7:** WO 02/26993 A (LIAW HUNGMIN JAMES ; MAO WEIYING (US); YANG YUEQIN (US); BRADSHAW JIL) 4 April 2002
- D8:** TODA KIYOSHI: "Theoretical and methodological studies of continuous microbial bioreactors." JOURNAL OF GENERAL AND APPLIED MICROBIOLOGY, vol. 49, no. 4, August 2003, pages 219-233
- D9:** ZENG A-P: "Continuous culture" 1999, IN: "MANUAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY" (ED. DEMAIN A L ET AL), 2ND ED., ASM PRESS, WASHINGTON

D1 and D2 were cited in the application.

2.2 NOVELTY (Art. 33(2) PCT)

- 2.2.1** The present application satisfies the criterion set forth in Article 33(2) PCT insofar as the subject-matter of **claims 1-33 and 35-38** is new in respect of the prior art as defined in the regulations (Rule 64(1)-(3) PCT).

- 2.2.2** The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of **claim 34** is not new in the sense of Article 33(2) PCT.

D1 discloses a threonine-producing sucrose-utilising transconjugant of *Escherichia coli* K-12 obtained by transfer of sucrose assimilation determinants from *E. coli* strain H155 to M-1 (**D1** figure 2 and page 124 line 12-27). In view of what is disclosed of DSM 16293 on page 27 line 15 - page 27 line 5 of the current description, it is assumed that no difference exists between the strain DSM 16293 and strains as disclosed in **D1**. Therefore, it is concluded that **D1** anticipates novelty of the subject-matter of **claim 34**.

2.3 INVENTIVE STEP (Art. 33(3) PCT)

- 2.3.1** Even if the subject-matter of **claim 34** referring to strain DSM 16293 would be acknowledged as novel, it still lacks inventivity. In view of **D1** (see passages cited above) and **D2** (page 257 line 14-20), the skilled person would be motivated to transfer sucrose assimilation determinants to a threonine-producing strain, e.g., from a strain such as H155 as has been done for DSM 16293. In the absence of any proven unexpected effect of such a transfer, no inventive step can be acknowledged.
- 2.3.2** **D1** and **D2** can independently be regarded as being the closest prior art to the subject-matter of independent **claim 1** and disclose a process for the preparation of L-threonine using bacteria of the Enterobacteriaceae family (*Escherichia coli*) using a batch fermentation process. The subject-matter of **claim 1** differs in that a continuous process is referred to wherein the concentration of the C-source which is fed to the continuous culture is not more than 30 g/l.
- 2.3.3** The problem to be solved by the subject-matter of **claim 1** may therefore be regarded as to provide a further or improved process for the fermentative preparation of L-threonine. The solution as proposed in **claim 1** of the present application can be summarised as the provision of a continuous process for the fermentative preparation of L-threonine with the C-source concentration in the continuous culture being adjusted to not more than 30 g/l.

- 2.3.4** This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons:
- 2.3.4.1** Continuous fermentation processes wherein the C-source concentration is limited are well known in the art. E.g., **D3** and **D4** disclose a continuous process for the production of lysine by coryneform bacteria wherein the C-source concentration is maintained at levels well below 30 g/l (cf. **D3** column 1 line 33 - column 3 line 40, examples 1, 2, 4 and 6-9, figure 6 and claim 1, **D4** page 2 line 1 - page 3 line 46, examples 1 and 2, claims 1, 3 and 4, figure 2). **D5** discloses a continuous process for the fermentative production of tryptophan by *E. coli* cells, wherein glucose as C-source is limited to below 30 g/l (cf. figure 12, paragraph 3.3). C-source limitation in processes for the fermentative production of threonine by *E. coli* strains is disclosed in **D6** (cf. paragraphs [0015] to [0017], examples 2 and 3; see also **D7** page 39 line 21 - page 40 line 27 describing optional production of threonine by continuous fermentation of *E. coli* cells with C-source limitation). General aspects of continuous processes are disclosed in **D8** and **D9**.
- 2.3.4.2** *In the absence of experimental proof, e.g., in the form of an example, for the achievement of any unexpected technical effect connected to a substantiated surprising advantage of applying a continuous process with C-source limitation for the production of L-threonine by microorganisms belonging to the family of Enterobacteriaceae, it is considered that the process as claimed merely represents an application of a well-known alternative production method connected to well-known general advantages, which the skilled person would consider to apply in order to solve the problem posed. Hence, no inventive step can be acknowledged.*
- 2.3.5** In the absence of any experimental proof, e.g., in the form of an example, it is unclear where any invention in the subject-matter of any dependent claim may lie. Subject-matter covered by these claims seems to follow directly, e.g., from documents cited in the description or on the ISR, or is considered to fall within the general knowledge and ability of the skilled person. Hence, at present, no inventivity of any of the dependent **claims 2-33 and 35-38** can be acknowledged either.

2.3.6 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of **claims 1-38** does not involve an inventive step (Rule 65(1)(2) PCT).

2.4 INDUSTRIAL APPLICABILITY (Art. 33(4) PCT)

2.4.1 The subject-matter of **claims 1-38** satisfies the criterion set forth in Art. 33(4) PCT in conjunction with Rule 5(vi) PCT with respect to industrial applicability.